European Journal of Medicinal Chemistry 120 (2016) 329–337

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Synthesis, characterization and *in vitro* evaluation of amphiphilic ion pairs of erythromycin and kanamycin antibiotics with liposaccharides

Rosario Pignatello [a, b, *], Pavla Simerska [c], Antonio Leonardi [a], Adel S. Abdelrahim [c], Giulio Petronio Petronio [d], Virginia Fuochi [e], Pio Maria Furneri [e], Barbara Ruozi [f], Istvan Toth [c, g, h]

[a] Section of Pharmaceutical Technology, Department of Drug Sciences, University of Catania, viale A. Doria, 6, 95125 Catania, Italy
[b] NANO-i – Research Centre on Ocular Nanotechnology, University of Catania, 95125 Catania, Italy
[c] The School of Chemistry and Molecular Biosciences (SCMB), The University of Queensland, Brisbane, Queensland 4072, Australia
[d] IRCCS San Raffaele Pisana, BioBIM – Multidisciplinary Interinstitutional BioBank, Rome, Italy
[e] Section of Microbiology, Department of Biomedical and Biotechnological Sciences – BIOMETEC, University of Catania, via Androne, 81, 95124 Catania, Italy
[f] Nanomedicine Group, Te.Far.T.I. Center, Department of Life Sciences, University of Modena and Reggio Emilia, 41124 Modena, Italy
[g] The School of Pharmacy, The University of Queensland, Brisbane, Queensland 4072, Australia
[h] The Institute for Molecular Biosciences, The University of Queensland, Brisbane, Queensland 4072, Australia

ARTICLE INFO

Article history:
Received 6 October 2015
Received in revised form
30 April 2016
Accepted 30 April 2016
Available online 6 May 2016

Keywords:
Erythromycin
Kanamycin A
Lipoamino acid
Charged liposaccharide
Amphiphilicity
Antimicrobial activity

ABSTRACT

The hydrophilic ion paring strategy (HIP) is a method explored to improve the cell/tissue uptake of poorly adsorbed drugs and to optimize their physico-chemical characteristics. In this context, we here describe the synthesis of some ion pairs of two model cationic antibiotics, erythromycin (ERY) and kanamycin A (KAN), with liposaccharides having different levels of lipophilicity and charge. The formation of drug-liposaccharide complexes was confirmed by Fourier transform infrared spectroscopy (FT-IR), differential scanning calorimetry (DSC) and powder X-ray diffraction (PXRD) analysis.

The effect of the amphiphilic liposaccharide moieties on the antimicrobial activity of ERY and KAN was assessed by measuring the minimal inhibitory concentration (MIC) of the compounds against a panel of bacterial strains that were susceptible or resistant to the parent antibiotics. The ion pairing did not depress the *in vitro* antibiotic activity, although no lowering of MIC values was registered. The experimental findings would motivate the future investigation of this ion pairing strategy in drug design, for instance allowing improvement of the encapsulation efficiency of hydrophilic antibiotics in lipid-based nanocarriers, or changing their *in vivo* biodistribution and pharmacokinetic profile.

1. Introduction

Administration of many hydrophilic drugs is often limited to the parenteral routes for various reasons, including poor penetration of the intestinal mucosa and/or binding to the gastrointestinal tract. However, oral delivery remains the preferred mode of drug administration due to better patient compliance. For this reason, pharmaceutical research has focused on the development of methods that improve oral drug bioavailability. Several strategies have been employed, such as the use of surfactants, penetration enhancers, mucoadhesive materials, drug delivery systems, prodrug formation and selective chemical modifications [1–5].

One of the main strategies for improving gastrointestinal absorption is increasing the lipophilicity of the drug [6]. Recently, many studies have reported the influence of including enhancers (e.g. bile salts and fatty acids) on drug adsorption in the intestine, their permeability through different biological membranes, and the use of lipidation to promote passive diffusion [7]. The addition of a safe and efficient absorption enhancers to the conventional oral dosage form of a drug is easier and cheaper than developing a new drug or prodrug.

Lipoamino acids (LAAs) are amino acids with a lipophilic alkyl side chain that can easily be coupled with peptide and/or carbohydrate moieties [8]. LAA-modified compounds not only improve absorption but also affect the physico-chemical and

* Corresponding author. Department of Drug Sciences, University of Catania, viale A. Doria, 6, 95125 Catania, Italy.
E-mail address: r.pignatello@unict.it (R. Pignatello).

http://dx.doi.org/10.1016/j.ejmech.2016.04.074
0223-5234/

pharmacokinetic properties of the drug [9,10]. LAAs have been shown to improve absorption, penetration, and/or oral bioavailability of several therapeutic agents [8,11–14], including some antibiotics [15–18].

As an alternative to covalently linking LAAs with the drug molecules, LAAs were shown to form amphiphilic ion pairs with antibiotics [e.g. erythromycin (ERY), tobramycin and kanamycin A (KAN)], without impairing their antimicrobial spectrum of activity [19–21]. However, the solubility of these drug-LAA ion pairs in aqueous media was limited by the high hydrophobic character of the LAA moieties [19–21]. For instance, the KAN-LAA ion pairs [21] possessed a very low aqueous solubility, that can at least in part explain their poor *in vitro* antibacterial activity.

Liposaccharide structures made from glycosylated LAAs have been used to moderate LAA hydrophobicity and possibly take advantage of the active glucose transport systems [7]. Liposaccharides consist of a lipophilic tail (LAA) and a hydrophilic head (carbohydrate), thus form amphiphilic moieties, which modulate both the aqueous solubility and the lipophilicity of a drug/liposaccharide conjugate [7]. Some of us recently optimized their synthesis and demonstrated that the liposaccharides were non-toxic at the concentrations used for oral delivery [22,23]. Furthermore, liposaccharides have been used to increase drug partitioning between *n*-octanol and water, suggesting that they enhanced the absorption of hydrophilic drugs that would have poor availability via the oral route [23,24].

On these premises, in the present work we assessed the effect of including a sugar moiety in LAA ion pairs with two model antibiotics (ERY and KAN) on the *in vitro* antibacterial activity of the drugs.

ERY (Fig. 1) is a clinically relevant macrolide antibiotic active against Gram-positive cocci, such as streptococci, and Gram-negative cocci. It is also active against *Bordetella pertussis*, *Brucella* spp, *Mycoplasma pneumonia,* and intracellular microorganisms like *Chlamydia*. ERY, after its binding with 50S ribosomal subunit, inhibits protein synthesis by stimulating the dissociation of peptidyl-tRNA from ribosomes [25]. Gram-negative rods, specially enteric species, are resistant to ERY because their outer membrane affects its permeability, thus reducing the intracellular concentration of the antibiotic [26,27]. According to Biopharmaceutics Classification System (BCS), ERY belongs to class 4 drugs, with low solubility and low permeability; for these reasons, it is used in clinics in the form of salts and esters, with variable physico-chemical and pharmacokinetic profiles [28].

KAN (Fig. 1) is an aminoglycoside antibiotic isolated from *Streptomyces kanamyceticus*. It is comprised of kanamycin A (the major component), and kanamycin B and C (minor components) [29]. KAN interferes with mRNA translation by binding irreversibly to four nucleotides of 16S rRNA and a single amino acid of protein S12 [30]. The penetration of aminoglycosides through the cell membrane is an aerobic, energy-dependent process [30]; consequently KAN is effective against bacteria with aerobic metabolism and has no efficacy against anaerobe as well as decreased activity facultative anaerobes such as streptococci and enterococci [31]. This limitation is often overcome by co-administering a bacterial cell wall synthesis inhibitor, such as a penicillin or vancomycin [32].

In this study, to assess the possibility of applying the ion pairing strategy to antibacterial drugs, two model positively charged (cationic) antibiotics, ERY and KAN, and three liposaccharides with different lipophilicity and charge characteristics (Fig. 2) were prepared by co-evaporation of equimolar water/ethanol co-solutions. The ion pair formation and molecular structure of the synthesized drug-liposaccharide complexes were characterized by Fourier transform infrared spectroscopy (FT-IR) analysis, differential scanning calorimetry (DSC) and powder X-ray diffraction (PXRD) analysis.

The effect of ion pairing with amphiphilic liposaccharide moieties on ERY and KAN antibacterial activity was assessed *in vitro* by evaluating the minimal inhibitory concentration (MIC) of the constructs against two representative Gram-negative species (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853), and three Gram-positive species freshly isolated, (*Enterococcus faecalis*, *Lactobacillus casei*, and *Streptococcus pyogenes*).

2. Results and discussion

2.1. Design and synthesis

Previous investigations showed that the electrostatic combination of cationic compounds, including ERY and KAN, with LAAs improved their antimicrobial activity [19–21]. However, the solubility of the obtained drug-LAA ion pairs in aqueous media was limited by the hydrophobicity of the LAA moiety. In particular, KAN-LAA ion pairs showed amphiphilic characteristics with limited solubility in water and aqueous buffer solutions [21].

On this basis, in the present work we assessed the effect of

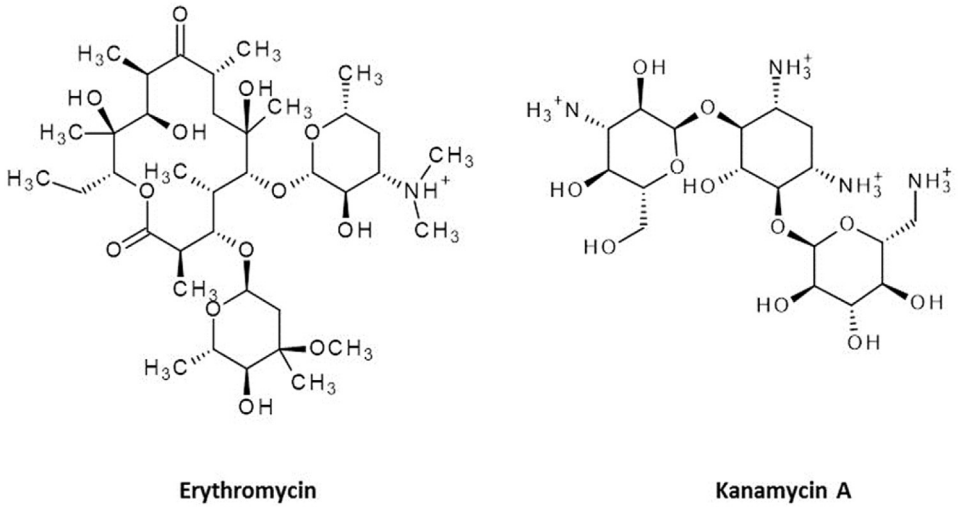

Fig. 1. Structures of erythromycin (ERY) and kanamycin A (KAN).